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GRAFT INCOMPATIBILITY IN *PINUS CONTORTA*

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ABSTRACT

The characteristic symptom of incompatibility in 2- and 3-year-old lodgepole pine grafts was recessed union area that resulted from the unequal growth of adjacent union and nonunion xylem cambia. Xylem growth in union areas stopped prematurely or grew at an abnormally slow rate during the summerwood period, while scion and stock cambia on both sides of the union zones grew normally. Union zones were sunken below the level of surrounding xylem cells. New xylem growth by union tissues the following spring appeared to be normal, but again growth terminated abnormally early or was slowed during the summerwood period, and even deeper recessed areas resulted. Finally, compression girdles of necrotic cells formed within recessed bark areas. The presence of chlorotic needles and scion overgrowths proved to be accurate indicators of incompatibility and can be used as criteria to rogue problem grafts from lodgepole pine seed orchards. Abnormally short needles may also be a symptom of incompatibility. Compatible grafts had normal growth in both union and nonunion tissues, so no recessed areas or external distress symptoms were seen.

Keywords: Grafting, lodgepole pine, *Pinus contorta*, vegetative propagation (nursery).

INTRODUCTION

Little grafting of lodgepole pine (*Pinus contorta* Dougl.) was done in the United States until Weyerhaeuser Company started a grafting program in 1972 for a seed orchard near Klamath Falls, Oregon. They have made several thousand grafts since 1972; and 2 to 3 years after grafting, approximately 10 percent of the grafts exhibited external symptoms of incompatibility. A similar incidence of incompatibility was found in lodgepole pine grafts made in New Zealand.^{1/} The most characteristic symptoms of incompatibility in both the Weyerhaeuser and New Zealand grafts were scion overgrowths at the union and chlorotic needles. Weyerhaeuser personnel recognized the problem as a potential hindrance to their tree improvement program and volunteered some of their graft unions for study. This paper presents the results of an anatomical examination of those 2- to 3-year-old *Pinus contorta* grafts.

MATERIALS AND METHODS

Grafting was done by Weyerhaeuser Company seed orchard personnel in the spring of 1972 and 1973. Top-cleft grafts were made on native seedlings found growing near selected plus-trees. It is likely that some stock trees were siblings of the scion clones grafted upon them. Immediately after

grafting, the grafted trees were dug and put into 4- to 7-gallon containers, then hauled to the seed orchard site where they were kept in the containers for the next 2 to 3 years. A regular water and fertilizer schedule was followed.

Needle chlorosis, reduced needle elongation, and scion overgrowth at the graft union appeared on a number of the grafts 2 to 3 years after grafting. In 1974 Weyerhaeuser personnel collected a sample of 40 graft unions that appeared to be healthy or compatible and others that appeared to be incompatible. Their selection was based solely on the previously mentioned union and needle chlorosis symptoms. Collected unions were stored in 50-percent alcohol immediately after cutting from stocks. The unions were cut into cross sections 25 μ m thick with a sliding microtome, stained with safranin-0 and fast green, and made into permanent microscope slides.^{2/}

RESULTS AND DISCUSSION

Comparison of the phenotypically selected incompatible grafts with healthy grafts showed incompatible grafts to have recessed xylem areas where stock and scion xylem tissues were contiguous (figs. 1-3). Healthy grafts showed little or no recessed areas in union zones

^{1/} Thulin, I. J. 1975. Personal communication dated May 1, 1975. For. Res. Inst., N. Z. For. Serv., Rotorua, N. Z.

^{2/} Copes, Donald L. 1967. A simple method for detecting incompatibility in 2-year-old grafts of Douglas-fir. U.S. Dep. Agric. For. Serv. Res. Note PNW-70, 8 p. U.S. Dep. Agric. For. Serv., Pac. Northwest For. and Range Exp. Stn., Portland Ore.

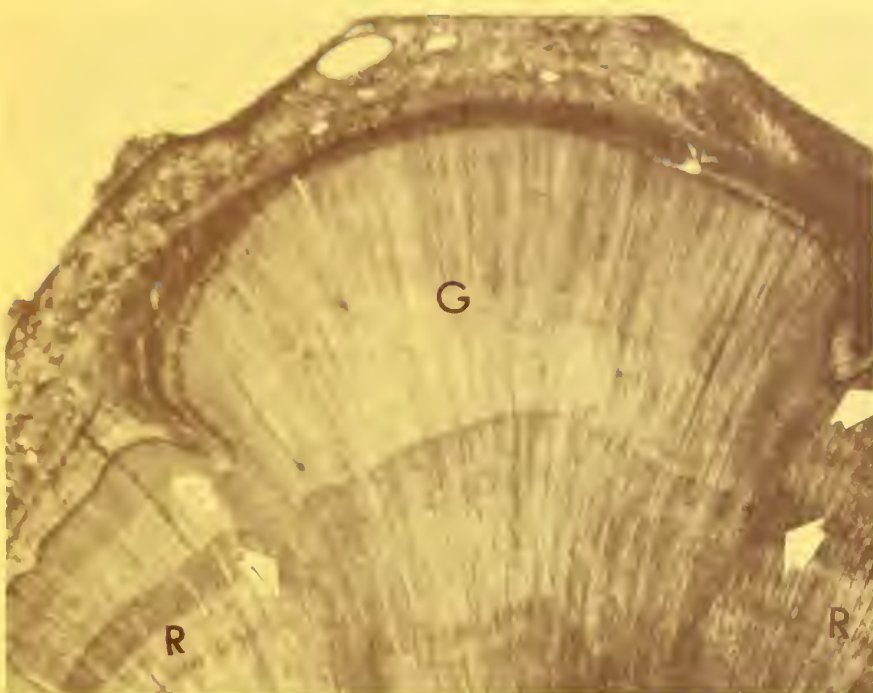


Figure 1.--A typical incompatible lodgepole pine graft union is pictured in cross section at 15X magnification. Recessed union areas are identified at the arrows (arrow 1 = 1973 recessed xylem and arrow 2 = 1974 recessed xylem). Recessed areas were deeper at the end of the third year than at the end of the second year. (G, scion; R, rootstock.)



Figure 2.--The recessed union area No. 1 from figure 1 is pictured in cross section at 100X magnification. This sunken area formed the second summer after grafting and was the first indication that the graft was incompatible. No abnormal cell types preceded the first growth stoppage, and little change in cell arrangement or type occurred when growth resumed the following spring. (G, scion; R, rootstock.)



Figure 3.--Sunken xylem and bark area No. 2 from figure 1 is pictured in cross section at 100X magnification. This abnormality occurred in the same union zone as that in figure 2, but at the end of the third year rather than the second year. The depth of the recessed area was much greater at the end of the third year than at the end of the second year. Compression of bark cells within the sunken areas had begun. (G, scion; R, rootstock.)

(figs. 4-6). Depth of the recessed areas in incompatibles varied within the two to four union zones where stock and scion cells were contiguous. Recessed areas were usually deeper in 3-year-old grafts than in the 2-year-old grafts.

The last formed tracheids at the bottom of each recessed area indicated that xylem formation ceased or slowed down after one-half to three-quarters of each year's summerwood increment had formed. No wound cells or other signs of trauma were evident before the first growth stoppage or slow down (fig. 3). Xylem initials in union zones formed fewer xylem cells than did stock and scion cells adjacent to the union areas where

normal growth continued. The unequal growth between adjacent cells resulted in characteristic sunken or recessed areas in the annual growth rings seen in cross sections (fig. 1).

Incompatible grafts were not noticeably different from compatible grafts through the first year. Some recessed xylem areas were found in the first annual ring of 2- and 3-year-old grafts, but ordinary grafting imperfections, such as slight errors in positioning of the cut stock and scion cambial surfaces at time of grafting, made it difficult to differentiate between incompatible and technique-caused deformities at the end of the first year. Technique-caused irregularities were

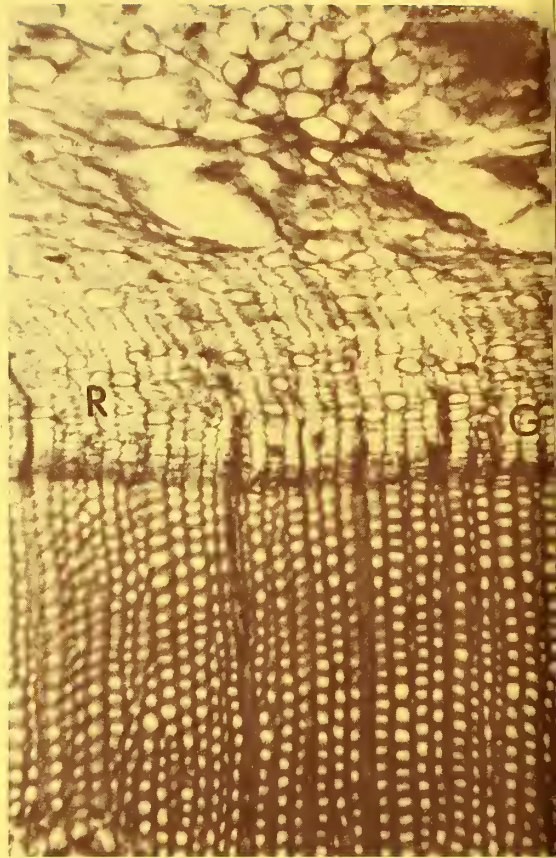


Figure 4.--A compatible lodgepole pine graft is shown in cross section at 15X magnification. No recessed areas were visible in any of the four union zones. Union zones indicated at the arrows (white = 1972, black = 1973 and 1974). Slight irregularities in the annual ring occurred at the end of the first year in several union zones; but normal growth the second and third years compensated for the sunken areas, so a round xylem cylinder was formed. (G, scion; R, rootstock.)



Figure 5.--Union zone No. 1 from figure 4 is shown in cross section at 100X magnification. No signs of abnormal growth were seen. Cell types before and after the annual ring were normal. (G, scion; R, rootstock.)

Figure 6.--The xylem and bark areas of union zone No. 1 of figure 4 are shown in cross section at 100X magnification. No sign of a recessed area was visible 3 years after grafting. The cambia of stock and scion appeared normal, and there were no symptoms of graft incompatibility. (G, scion; R, rootstock.)



reduced or eliminated during the second year. At that time the cambial zone was much more circular when seen in cross section and detection of recessed union areas was much easier. Detection after 3 years was even easier than at the end of the second year because deeper invaginations had formed (figs. 2 and 3).

Recessed areas of graft unions were not visible externally (fig. 1). Phloem growth may have been stimulated where xylem growth was abnormal since the bark areas of many union areas had a profusion of phloem cells in radial files. Increased phloem growth physically compensated for the lack of xylem cells in recessed areas.

Little necrosis was observed in bark areas of 2-year-old incompatible grafts, but a number of 3-year-old grafts had isolated islands of necrotic phloem and cortex. Necrosis appeared to be preceded by cell dilation and was restricted to union tissues (fig. 7). Cell rejection in lodgepole pine was very different from Douglas-fir grafts, where a possible hypersensitive type of reaction caused extensive suberization and necrosis of contiguous stock and scion cells.^{3/} Necrosis in

^{3/} Copes, Donald L. 1970. Initiation and development of graft incompatibility symptoms in Douglas-fir. *Silvae Genet.* 19(2-3): 101-107.

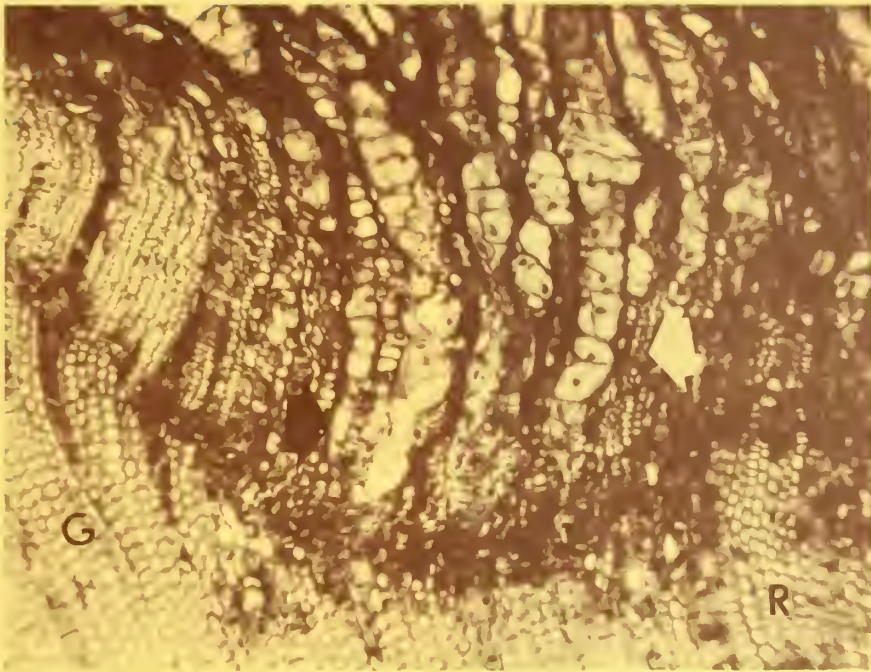


Figure 7.--Dilated ray cells in bark tissues of an invaginated union zone are shown in cross section at 100X magnification. The union cells were larger than similar cells in nonunion locations. Greatly enlarged cells in unions were necrotic in many 3-year-old grafts. (G, scion; R, rootstock.)

lodgepole pine grafts appeared to be more of an indirect effect, resulting from cell crowding or compression in recessed areas rather than from between-cell interactions.

Xylem growth began each spring without the formation of large wound areas that characterize incompatible Douglas-fir grafts (see footnote 3). No abnormal cells were formed the next spring, so the cambia in the union zones had merely become dormant during the summer-wood period and had not been physically changed. When the factors limiting growth were removed or reduced the following spring, the union areas grew normally until the limiting conditions recurred. Recurrence of the same phenomenon compounded the problem caused by the previous year's reduced growth and

even deeper recesses formed (figs. 2 and 3). Bark and cambial areas within deeply recessed areas became compressed and isolated. Necrosis of cells in compressed areas resulted in a girdle zone of dead tissue that separated stock and scion. Girdled trees had reduced needle growth, chlorotic needles, and scion overgrowths and would have died within several years if they had not been sacrificed for study.

The recurrence of premature growth termination the third year was often preceded by the proliferation of ray and axial parenchyma in recessed xylem areas. Axial parenchyma cells, resembling epithelial cells which normally surround resin canals, were present in great numbers but with no apparent organization (fig. 8).

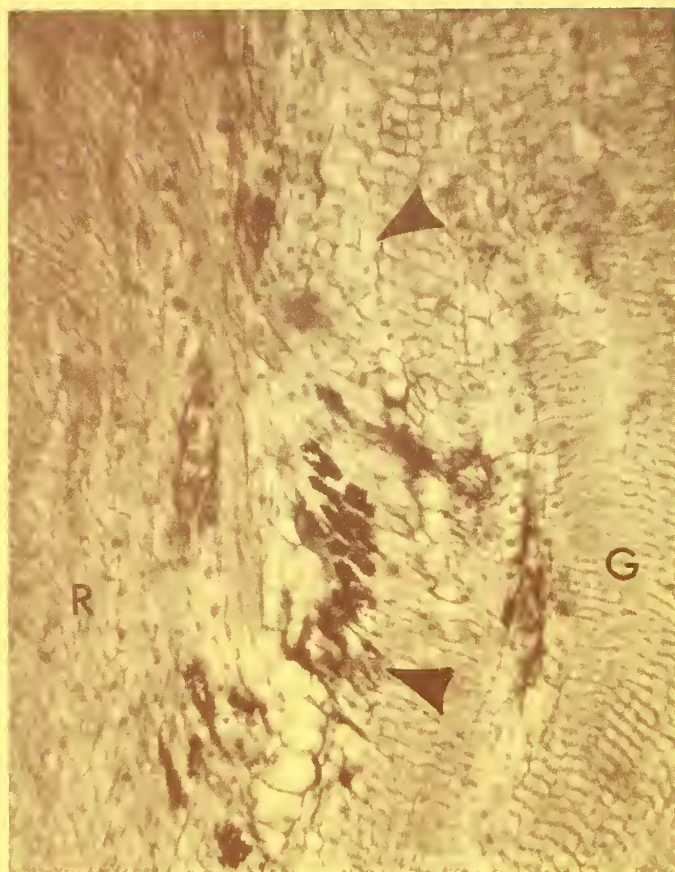


Figure 8.--An abundance of axial parenchyma was found in the xylem of union zones seen in cross section at 100X magnification. The occurrence of masses of disorganized axial parenchyma cells was fairly common in union areas of 3-year-old incompatible grafts but less frequent in 2-year-old grafts. (G, scion; R, rootstock.)

Field identification of incompatible and compatible grafts by color and length of needles and by external union appearance was accurate. Needle chlorosis and scion overgrowth were incompatibility symptoms. Only one phenotypically selected incompatible graft lacked the characteristic recessed xylem areas. Also only one phenotypically selected compatible graft was found to be incompatible. The latter was a 2-year-old graft, and it may not have had adequate time for external symptoms to become apparent. Since only 2 incorrect estimates out of 40 were made, the correlation between phenotypic selection and internal structure was good. Phenotypic roguing of incompatible grafts in the United States

can be done at the end of the second or third year. Good results have been obtained in New Zealand when roguing was done at the end of the first year (see footnote 1). Accurate detection of incompatibility 1 year sooner than in Oregon may be due to the faster growth in New Zealand. With accurate external roguing, anatomical testing is not necessary.

RESEARCH SUMMARY

Microscopic study of lodgepole pine grafts showed incompatibility resulted in unequal xylem growth in union and non-union areas. In incompatible grafts, xylem growth ceased abnormally early or grew very slowly during the summerwood

period, while contiguous non-union xylem areas continued normal growth. Unequal growth of adjacent cells resulted in recessed union areas that completely encircled the graft union. Compatible grafts had nearly equal growth in union and nonunion areas and did not form

recessed xylem areas. Phenotypic roguing of incompatible grafts is accurate when done 2 or 3 years after grafting. Useful roguing criteria are needle chlorosis and scion overgrowths. Anatomical tests for incompatibility are not necessary.

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